Chapter 2

Literature Review

2.1 General

This chapter presents a detailed review of MFC technology with its design, history and working principle. The review discusses various parameters affecting the efficiency of MFC. It also discusses the utilization of wastewater for the generation of electricity. It discusses various microorganisms responsible for the activity and a detailed review on the mechanism of transfer of electrons from bacteria to anode.
2.2 MFC Technology

The history started with the relationship between biology and electricity when Galvani in 1791 observed electric current generation with twitching of frog’s leg. Fifty years from then, in 1839, Grove created first fuel cell. In his experiment, Grove reversed the process of electrolysis of water, where hydrogen and oxygen were also combined back to water and electricity produced during the process as described by Ieropoulos et al. (2005).

In 1911, Prof. Potter in botany at the University of Durham, UK, described the electricity production gained from cultures of yeast and *Escherichia coli*. It was the first time that biological process was observed producing bioelectricity (Potter 1911). Further progress made by Cohen in 1931 that could produce more than 35 V through half fuel cells connected in series as reported by Singh and Songera (2012). Palmore and Whitesides (1994) reported the increased demand of biological fuel cell during 1960s due to the space race as the USA space program was interested in this technology.

Rao and Drake (1969) showed that using platinum as the working electrode, glucose can be used as fuel to produce power and in 1976 they identified the clear principle of the biological fuel cell (Rao et al. 1976). The name and concept of MFCs was first explored from 1980s (Roller et al. 1984). The concept of combining wastewater treatment and MFCs was introduced in 1991(Habermann and Pommer 1991).

2.2.1 MFC Design

Broadly two MFC designs are available: (a) single chambered MFC and (b) two chambered MFC. The basic difference between the two depends on the number of reservoirs. In single chambered MFC (Figure 2.1), both electrodes are in the single chamber. The electrons generated at anode by bacteria are accepted by oxygen at cathode. The disadvantage is diffusion of oxygen at anode inhibiting bacteria from transferring electrons to anode, thereby, reducing the efficiency of electricity generation (Aziz et al. 2013). Liu and Logan (2004) reported a maximum of 0.49 W/m² of power production using single chambered MFC.
In two chambered MFC (Figure 2.2), the reservoirs are separated by proton exchanger either as salt bridge, cation exchange membrane or proton exchange membrane (Das and Mangwani 2010). The anodic chamber contains feed, source of bacteria and anode. The cathodic chamber contains oxygen as catholyte which can be replaced by an electron accepting chemical such as potassium ferricyanide or potassium permanganate. The H-shaped two chambered MFC is widely used design (Aziz et al. 2013). Rabaey et al. (2003) obtained a maximum power output of 4.31 W/m² using a two chambered MFC and ferricyanide as a cathode electrolyte, with 81% of electron transfer efficiency.
Apart from the variations in the design and electron accepting entity in cathodic chamber, components such as electrodes, wiring, external resistances, feed and bacterial inoculum remains constant.

### 2.2.2 Working of MFC

In MFCs, bacteria at the anode metabolize biodegradable matter for example glucose. During the metabolism they generate electrons and protons. Electrons are transferred to cathode through external resistance; simultaneously protons are transferred to proton exchangers, thus completing the circuit and generating electricity (Figure 2.3) (Oh and Logan 2006; Oliveira et al. 2013; Rabaey and Verstraete 2005). Thus if biodegradable matter is available in abundance then microbes will continuously generate and transfer electrons and protons to anode resulting in continuous generation of electricity.

As an example, potential of glucose and acetate in generating electrons given by Fornero et al. (2010) is shown as follows:

\[
\begin{align*}
C_6H_{12}O_6 + 6H_2O & \rightarrow 6CO_2 + 24H^+ + 24e^- \\
CH_3COOH + 2H_2O & \rightarrow 2CO_2 + 8H^+ + 8e^-
\end{align*}
\]

![Figure 2.3 Working Principle of MFC](image)
2.2.3 MFC Materials

The main challenge in the construction of MFC is use of material which can increase the power production. The next challenge is to minimize the cost as MFCs are scalable systems. The three main components of MFCs are anode, cathode and membrane.

One of the primary and essential factors is electrode material because it is related to the interaction with bacteria through anode and reaction between electron and proton with high reduction potential entity such as oxygen at cathode. Since the anode deals with bacterial growth, they should have good biocompatibility, high surface area for the growth deposition and the development of biofilm along with high conductivity, chemical stability especially in wastewater, good adaptability, stability at different temperatures and pH, resistance to biofouling and should have low cost. Carbon materials satisfy almost all criteria and hence they are preferred as anode. Different forms of carbon materials are available such as cloth, fibres, and graphite plates, rods and granules (Hu 2009).

The required properties of cathode are very similar to anode except for the fact that cathodes may not be biocompatible. However, when bio-cathodes are used in MFCs then biocompatibility should be taken into consideration. In case of oxygen used at cathode, the cathode material should have certain amount of platinum which acts as a catalyst for the reduction of oxygen. But platinum is a costly metal and can be easily poisoned by microbial by-products such as hydrogen sulphide (Logan 2008). Thus to reduce the cost, materials similar to anode are used for cathode which lack platinum and instead of oxygen potassium ferricyanide or potassium permanganate is preferred as they readily accepts electrons (You et al. 2006).

In case of two chambered MFCs, anodic and cathodic chambers are separated by proton exchanger with a basic function of selective transfer of proton from anode to cathode. The commonly used proton exchangers are salt bridge, cation exchange membrane (CEM) and proton exchange membrane (PEM). Considering the cost and the performance, ascending order will be PEM > CEM > salt bridge. Both PEM and CEM are permeable to gas but impermeable to water. This is another disadvantage apart from being costly is diffusion of
oxygen from cathodic chamber to anodic chamber reducing the efficiency of the system (Logan 2008). Yet, the diffusion is less than in case of single chambered MFCs.

The details of materials used to improve MFC performance is mentioned in section 2.2.5.1 and 2.2.5.3.

2.2.4 MFC as Wastewater Treatment Technology

The efficiency of conversion into electricity also depends on the components present in the waste matter. When we are talking about substrate or feed in MFC, the composition and the concentration becomes very important because it has been observed that the microbial community present at the anode and the power output in MFCs is influenced by the nature of feed. Pure chemical compounds such as glucose and other monosaccharides like xylose, arabinose, fructose, mannose, galactose (Catal et al. 2008a), lactate (Lanthier et al. 2008), acetate (Logan et al. 2007), propionate (Oh and Logan 2005), sucrose (Behera and Ghangrekar 2009), mannitol and sorbitol (Catal et al. 2008b) have been used as feed.

The abundance of organic waste in the form of wastewater makes this application attractive for large scale energy generation. Wastewaters from different industries can be used as feed such as beer brewery (Köroğlu et al. 2014), chocolate industry (Patil et al. 2009), domestic (Ren et al. 2014), food processing (Oh and Logan 2005), dairy (Elakkiya and Matheswaran 2013) and synthetic wastewater amended with pure compounds mentioned above (Pant et al. 2010).

MFCs capture energy in the form of electricity without any input of electricity and this makes it a promising technology. The approach of MFC is completely different than the traditional methods of aerobic and anaerobic degradation of wastewater. Kim and co-workers demonstrated the use of industrial wastewater sustained by starch for electricity generation in late 1990s. However, the output of energy was low leading to curiosity regarding the applicability of this technology. In 2004, MFC was used to treat domestic wastewater to practical levels with simultaneous electricity generation (Liu et al. 2004). This study was the turning point for the improvement amongst the operational parameters, though the power produced was considerably low (26 mW/m²) but very high compared to previous experiments.
The use of marine sediments in MFCs was already shown by Reimers et al. (2001) emphasizing on the utility of wide variety of substrates and microbial sources. Following these demonstrations, the primary goal was set to increase the efficiency of MFCs in order to make it scalable technology for the treatment of domestic, industrial and other types of wastewaters. Different types of wastewaters used as feed has been elaborated in Table 2.1.

<table>
<thead>
<tr>
<th>Wastewaters used as feed</th>
<th>Power produced</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Domestic</td>
<td>91 mW/m²</td>
<td>(Liu et al. 2011)</td>
</tr>
<tr>
<td></td>
<td>12.8 W/m³</td>
<td>(Ahn and Logan 2010)</td>
</tr>
<tr>
<td></td>
<td>91 mW/m²</td>
<td>(Liu et al. 2011)</td>
</tr>
<tr>
<td></td>
<td>240 mW/m²</td>
<td>(Hays et al. 2011)</td>
</tr>
<tr>
<td></td>
<td>754 mW/m²</td>
<td>(Yu et al. 2012)</td>
</tr>
<tr>
<td></td>
<td>19.7 W/m³</td>
<td>(Ren et al. 2014)</td>
</tr>
<tr>
<td>Landfill leachate</td>
<td>0.344 W/m³</td>
<td>(Puig et al. 2011)</td>
</tr>
<tr>
<td></td>
<td>11 A/m²</td>
<td>(Özkaya et al. 2013)</td>
</tr>
<tr>
<td></td>
<td>0.699 W/m³</td>
<td>(Ganesh and Jambeck 2013)</td>
</tr>
<tr>
<td></td>
<td>0.824 W/m³</td>
<td>(Damiano et al. 2014)</td>
</tr>
<tr>
<td></td>
<td>0.78 W/m³</td>
<td>(Shan et al. 2011)</td>
</tr>
<tr>
<td>Brewery</td>
<td>4.1 W/m³</td>
<td>(Zhuang et al. 2012)</td>
</tr>
<tr>
<td></td>
<td>24.1 W/m³</td>
<td>(Wen et al. 2010a)</td>
</tr>
<tr>
<td></td>
<td>0.83 W/m³</td>
<td>(Wen et al. 2010b)</td>
</tr>
<tr>
<td>Dairy</td>
<td>20.2 W/m³</td>
<td>(Mardanpour et al. 2012)</td>
</tr>
<tr>
<td></td>
<td>3.2 W/m³</td>
<td>(Elakkiya and Matheswaran 2013)</td>
</tr>
<tr>
<td></td>
<td>1.1 W/m³</td>
<td>(Venkata Mohan et al. 2010)</td>
</tr>
<tr>
<td>Distillery</td>
<td>0.457 W/m³</td>
<td>(Kaushik and Chetal 2013)</td>
</tr>
<tr>
<td></td>
<td>0.429 W/m³</td>
<td>(Samsudeen et al. 2014)</td>
</tr>
<tr>
<td></td>
<td>0.124 W/m³</td>
<td>(Huang et al. 2011)</td>
</tr>
</tbody>
</table>

Though the energy extracted through wastewater is not sufficient to power any city, it has enough potential to run a waste treatment plant.

2.2.4.1 Anaerobically Digested Distillery Wastewater

Water pollution is a major problem in India and as mentioned, distilleries are prime polluting industries. Treatment of high COD distillery wastewater is a challenge. It is generally treated with physico-chemical methods such as coagulation, adsorption and reverse osmosis. High
cost and tremendous sludge production are major inhibitions in the use of these methods. The alternate method of treating distillery wastewater is biological. These include aerobic degradation, anaerobic digestion, anaerobic filters, up-flow anaerobic sludge blanket reactors (Jain et al. 2002). Anaerobic treatment is preferred because of low sludge production. Amongst the anaerobic treatments, anaerobic digestion results in the formation of biogas – an utilizable form of fuel and hence preferred. Along with the fuel, it stabilizes the waste, and destroys viral and bacterial pathogens (Chen et al. 2008). However, the wastewater even after anaerobic digestion, that is ADDW, has a potential to be a pollutant due to the accumulation of recalcitrant compounds and presence of melanoidins (Ghosh et al. 2002) and presence of high COD (Singh 2010).

Literature revealed the focus on decolourization and COD reduction of ADDW due to dark coloured melanoidins. Kumar et al. (1998) achieved maximum decolourization upto 71.5% whereas COD reduction was 90% using fungal cultures Coriolus versicolor and Phanerochaete chrysosporium. However, the decolourization and COD reduction was achieved in 12.5% (v/v) ADDW broth media. Ghosh et al. (2002) used two-stage bioreactor for the treatment of ADDW. They used bacterial species Pseudomonas putida U and Aeromonas strain Ema for decolourization and COD reduction studies. P. putida showed 60% colour and 44.4% COD reduction, however, Aeromonas sp. could not decolorize ADDW though the COD reduction was more than P. putida (66%). Similar studies achieved the decolourization over a range of 67% to 81% (Adikane et al. 2006; Mohana et al. 2007; Shayegan et al. 2005), whereas the COD reduction was 51% and 84% by Mohana et al. (2007) and Shayegan et al. (2005) respectively.

Biological treatments, using fungi such as Coriolus sp., Aspergillus sp., Aspergillus niger, Trametes versicolor, Phanerochaete sp., Phanerochaete chrysosporium, Coriolus versicolor, Coriolus hirsutus and Penicillium decumbes, yeasts such as Citeromyces sp., and certain bacteria such as Bacillus sp., Pseudomonas fluorescence, and acetogenic bacteria have been reported (Jiranuntipon et al. 2009).
2.2.5 Factors Affecting Performance of Microbial Fuel Cell

The performance depends on the efficiency of bacteria to transfer their electrons by metabolizing the organic matter to anode. It also depends on the electrochemical reaction at cathode (Rabaey and Verstraete 2005). Considering the above two, factors responsible for the affecting the performance of MFCs are type of microorganism, type of organic biodegradable matter or feed, pH and temperature of the system, electrode material, proton exchanger, internal and external resistance, catholyte, aeration in cathodic chamber (Du et al. 2007).

2.2.5.1 Electrode Materials

Electrode materials can be improved for better performing by MFCs. For example, platinum and platinum black electrodes are superior to graphite, graphite felt and carbon-cloth electrodes for both anodes and cathodes (Du et al. 2007). Schröder et al. (2003) reported use of platinized carbon cloth as anode and produced a current of 2 – 4 mA by using E. coli in a standard glucose medium, however, an unmodified carbon-cloth showed no current flow under the same operating conditions. The basic advantage of platinum is its higher catalytic activity for oxygen reduction than graphite materials and hence MFCs with platinum or platinum-coated cathodes shows higher power densities (Moon et al. 2006; Oh et al. 2004). But platinum material is very costly and hence graphite material is preferred for electrodes. Electrode modification is actively investigated by several research groups to improve MFC performances. Along with the anode material and its configuration, surface areas of electrodes represent an important parameter in MFCs, as the current output was found to increase with the increase in thickness of the anode bed and with the approximate anode area (Di Lorenzo et al. 2010). In particular, a three-dimensional anode would allow a greater surface area for microbial attachment. Graphite granules have been widely used as both anode and cathode material (Aelterman et al. 2006a; Heilmann and Logan 2006; Rabaey et al. 2005a). Several other type of three-dimensional anodes have been previously tested such as reticulate vitreous carbon, granular activated carbon, carbon foam and graphite brush electrodes (Chaudhuri and Lovley 2003; He et al. 2005; Logan et al. 2007). In general the power production has shown to increase with higher surface area materials (Chaudhuri and Lovley 2003).
2.2.5.2 pH and Electrolyte

Bacteria degrade the organic matter in anodic chamber resulting in the increase in acidity because of the bacterial fermentation under anaerobic conditions. Ideally, the rate of consumption of protons at cathodic chamber should be equal to the generation of protons at anodic chamber, however, if no buffer solution is used, there will be a pH gradient between the anodic and cathodic chambers (Du et al. 2007). The gradient is due to inefficient proton exchanger separating the two chambers. Proton transport through the membrane is slower than its production rate in the anode and its consumption rate in the cathode chambers (Gil et al. 2003). However, the pH difference increases the driving force of the proton diffusion from the anodic to the cathodic chamber, eventually forming a dynamic equilibrium. Proton availability to the cathode is a limiting factor in electricity generation. Increasing ionic strength by adding NaCl to MFCs also improved the power output, possibly due to the fact that NaCl enhanced the conductivity of both the anolyte and the catholyte (Jang et al. 2004; Liu et al. 2005b).

2.2.5.3 Proton Exchanger

As explained above, proton exchange system affects a power output of MFCs. Total three types of proton exchangers have been used and are salt bridge, CEM and PEM as mentioned in section 2.2.3. The salt bridge has very low cost as compared to other two. CEMs allow all the cations to pass through, reducing the efficiency of transfer of protons from anodic to cathodic chamber. Nafion, a product of DuPont and a type of PEM, is the most popular because of its highly selective permeability for protons. Researchers are attempting to look for less expensive and more durable substitutes; however, Nafion is still the best choice, even though it is very costly. However, researchers have observed the same side effect as of CEM in case of Nafion (Rozendal et al. 2006). In this sense, Nafion is not necessarily a proton specific membranes but actually cation specific membranes.

The ratio of PEM surface area to system volume is another important factor to improve the power output. The internal resistance of MFCs decreases with the increase of PEM surface area (Oh and Logan 2006). Min et al. (2005) compared the performance of a PEM and a salt bridge in an MFC inoculated with G. metallireducens. They found that the power output using the salt bridge was 2.2 mW/m² and it was lower than that achieved using Nafion. Park and Zeikus (2003) even used a porcelain septum made from china clay instead of Nafion. Behera
and Ghangrekar (2011) used earthen pots of different thickness as proton exchanger. They observed increase in internal resistance with increase in thickness. All membranes along with porcelain septum are prone to fouling especially if the fuel is municipal wastewater. Membrane-less MFCs are desired if fouling or cost of the membrane becomes a problem in such applications.

2.2.5.4 Catholyte

Oxygen is the most commonly used electron acceptor in MFCs. Power output of an MFC strongly depends on the concentration level of electron acceptors. Several studies indicated that DO was a major limiting factor when it remained below the air-saturated level (Gil et al. 2003; Oh et al. 2004). To provide high DO at cathodic chamber, aeration is required with additional input of energy. Also, if high DO is provided, the rate of oxygen diffusion toward the anode chamber is also increased. Thus, part of electrons are consumed directly by the oxygen instead of transferring them to the anode and the circuit (Pham et al. 2004). As mentioned in section 2.2.5.1, platinum catalyst is required when oxygen is used as catholyte. Due to high cost, researchers use potassium ferricyanide as the electron acceptor in the cathodic chamber. So far, reported cases with very high power outputs are 7200 mW/m², 4310 mW/m² and 3600 mW/m² using ferricyanide in the cathodic chamber (Oh et al. 2004; Rabaey et al. 2003; Schröder et al. 2003). Hydrogen peroxide solution (Tartakovsky and Guiot 2006) and potassium permanganate (Behera and Ghangrekar 2009) have also been used as the final electron acceptor in the cathodic chamber. In case of catholyte other than cathode, aeration in cathodic chamber may not be required.

2.2.5.5 Temperature

MFCs are strongly affected by temperature since changes in temperature affects the nature and distribution of the microbial community (Oliveira et al. 2013). Although research in this field has increased during the last years, there is no systematic information regarding the influence of temperature in MFCs. It has been observed that temperature is a crucial factor and positively affects the COD removal and electricity generation (Min et al. 2008; Wang et al. 2008a). This microbial activity can be analyzed in terms of biofilm development at the anode which has an impact on anodic bio-catalytic activity. Higher temperatures lead to a stable biofilm and MFC operation in a short period of time and consequently a higher performance. It was found that the biofilms process their maximum bioelectrocatalytic
activity at a temperature between 30°C and 45°C (Michie et al. 2011a; Michie et al. 2011b; Patil et al. 2010), and keeping this temperature range is advantageous to operate a MFC in order to achieve higher performances. Different electrogenic bacteria had different optimum temperatures and hence the temperature during the initial growth phase of biofilm determines the abundance of the different microbial species as well as their distribution within the biofilm. Once the biofilm is formed, the microbial species can adjust their metabolism functioning at different temperatures (Michie et al. 2011a). These findings are important for application of MFC for wastewater treatment. Use of higher temperatures in the startup processes is better for performance of MFCs. After that, MFCs can operate at lower temperatures decreasing the associated heating costs without a significant decrease on performance (Oliveira et al. 2013).

2.2.5.6 Resistance

Two types of resistances exist in MFCs, internal and external. Internal resistance is imparted due to the use of proton exchanger (Logan et al. 2006). Salt bridge has very high internal resistance followed by membranes (Min et al. 2005). The external resistance is applied externally to the circuit. Internal and external resistances together decrease the performance of MFCs. Because, resistance resists the flow of transfer of electrons and hence high resistance cause bacteria to transfer electrons slowly which is detrimental to the bacteria as transfer to anode is accountable for bacterial net energy gain (the mechanism is further discussed in section 2.3.1). A high external resistance results in a high cell voltages and low current. Theoretically, the optimal resistance for maximum power generation approximates the internal resistance (Logan et al. 2006).

The effect of external resistance on MFC behavior has been addressed through a number of studies (Gil et al. 2003; Jang et al. 2004; Liu et al. 2005a). Liu et al. (2005a) observed higher COD removal with the MFC operated under external resistance when compared to open circuit voltage (that is without resistance). Lyon et al. (2010) found that the external resistance was associated with changes in the bacterial community structure on the surface of anode.
2.3 Microbiology of Electricity Generation

The electron flow is integral to microbial metabolism. Bacteria metabolize organic substrates for energy. The energy gained is through electron transport chain (ETC) which involves transfer of electrons and generation of protons. If the electron acceptor is internal then it is called fermentation while the electron acceptor is external then it is called respiration. In MFCs, biodegradable matter is oxidized in the absence of oxygen and the electrons and protons generated by respiratory bacteria are transferred to anode; such bacteria are called “anode-respiring bacteria” or “exoelectrogenic bacteria” (Logan 2008).

2.3.1 Respiratory Oxidation

When the terminal electron acceptor is an electrode, the anodic oxidation becomes respiration. The electricity generation mechanisms are very similar to oxygen or nitrate respiration with the net products H₂O, CO₂ and ATP. Anode respiring bacteria is benefited from tricarboxylic acid (TCA) cycle and membrane bound ETC. When acetate, lactate and ethanol are present in the system, they can enter TCA cycle through pyruvate or acetyl CoA with maximum two enzymatic steps as shown in Figure 2.4. Different species of anodophilic bacteria can utilize them directly as electron donors (Bond and Lovley 2003; Kim et al. 1999a; Kim et al. 2007). On the other hand, glucose requires more enzymes to carry out glycolysis. Fermentative bacteria are highly specialized in glycolysis. Hence, though glucose oxidation is observed using pure cultures (Chaudhuri and Lovley 2003), glucose is fermented in mixed consortia (Freguia et al. 2008).

The ETC starts with generation of NADH, NADPH and FADH₂ in TCA cycle. Electrons then travel though flavoproteins, iron-sulphur proteins, quinones and series of cytochromes. ETC generates proton motive force (PMF) across the membrane. The PMF drives ATP generation by the process of oxidative phosphorylation through membrane bound ATP synthase enzyme.
The last electron transfer step at anode can occur directly via cytochrome, nanowires or via redox mediators instead of oxygen as shown in Figure 2.5 (Freguia 2010a).

**Figure 2.4** Schematic Representation of TCA Depicting the Entry of Bacterial Substrates Glucose, Pyruvate, Ethanol, Lactate and Acetate (Freguia 2010a)
2.3.2 Fermentation

Fermentation occurs when the electron acceptor is internal. The substrate is initially oxidized to intermediate metabolites which act as electron acceptors and get reduced to fermentation products with the generation of energy. Since MFC prefer anaerobes, fermentations are common in such environments. Not all bacterial species have the ability to use electrode as terminal electron acceptor and hence fermenters makes the environment extremely competitive for electrogenic bacteria to grow and produce electricity.

Due to absence of high potential electron acceptors, fermentation yields little energy as compared to respirations. Acetogenic fermentations can yield upto 4 ATPs per glucose molecule while most other fermentations yield 2 ATPs per glucose. Since, ATP generation in fermentation is less than respiration, fermenters outgrows respirators and hence fermenters have much higher substrate turnover. So, with the available substrate, anode respiring bacteria have lesser chance of growth and exploiting the property of electricity generation (Freguia 2010b).
2.3.3 Methanogens Compete For Fermentation Products

Methanogenesis is the last stage of anaerobic digestion. The conversion of substrate to methane is carried out exclusively by Archaea. The substrates for methanogenesis are very few but considering the relevance to MFC, they are acetate (acetoclastic) and hydrogen (hydrogenotrophic). In any given anaerobic environment with acetate or H₂, methanogens are bound to develop. Thus in MFCs, methanogens are also found on the anode surface. Methanogens and anodophiles are in competition for the substrate as any substrate getting converted to methane is a loss for anode respiring bacteria. In case of electrogenic bacteria, if the electron transfer is through direct contact with the anode then maintaining the close proximity and establishment of the contact with anode becomes constraint for their growth. In that case, methanogens are at an advantage over anodophilic bacteria (Freguia 2010b).

Acetoclastic methanoges are slow growers and require excess of acetate in the medium. Hence, at continuous optimal loading rate, methane production is continuous by hydrogenotrophic methanogens. As soon as the loading rate exceeds the optimal value, acetoclastic methanogen convert the acetate into methane. It is observed that, during the optimal loading rate operation, 15 - 25% of glucose is converted to methane whereas in the latter case, the percent is increased to 50 (Freguia 2010b).

Most anodophilic bacteria are aerophilic, that is, they thrive on the respiratory ETC, and are not affected by the presence of oxygen. While some are aerotolerant and may require temporary aerobic condition to trigger the electrogenic activity (Yamazaki et al. 2002). On the other hand, methanogens are strict anaerobes and are killed rapidly with slightest exposure to oxygen. Hence, frequent aeration at anodic chamber is thought to decrease the competition between them. However, in case of thick biofilm, oxygen may not reach the deeper layers where methanogens continue to grow and reduce the efficiency of electricity generation (Freguia 2010b).
2.3.4 Syntrophy between Fermenters and Anodophiles

In a mixed consortia growing as a biofilm, populations of fermenters and respiring bacteria compete for the substrate. Apart from being the competitors, the commensalism or synergism may exist. Studies by Freguia et al. (2008) and Lee et al. (2008) revealed that the competition for carbohydrates is won by fermentative microorganisms. In most cases, over 90% of the sugars are converted to fermentative products rather than electricity. Since, glucose fermentation to acetate generates highest ATPs, acetogenesis is a dominant metabolic process shown by microorganisms. Acetate is always the main fermentation product, with propionate and butyrate acquiring importance next to it. Lactic and alcoholic fermentations are generally barely competitive in mixed anodic biofilms (Freguia 2010b).

All fermentation products can be substrates for anodophilic respires. Most carbohydrates are generally first fermented in mixed anodic communities; these products are then oxidized to generate electricity in a syntrophic process. Acetate is the fastest organic compound to be converted to electricity by bacteria (Freguia 2010a), along with propionate (Bond and Lovley, 2003), butyrate (Liu et al. 2005a) and other common fermentation products such as lactate (Park and Zeikus 2003), ethanol (Kim et al. 2007) and formate (Ha et al. 2008) who have also shown to work well as substrates for anodophilic bacteria.

Fermentations that produce volatile fatty acids also generate hydrogen gas to some extent. Few anodophilic species have ability to utilize the H₂ very effectively (up to 15%) through lithoheterotrophic fermentation (Freguia et al. 2008). Some fermenters can skip hydrogen production and transfer electrons directly to anode via soluble mediators such as hydroquinones (Wang et al. 2008b). Such mediators are used for electron transfer by other anodophiles as well, where they could insert into ETC and generate ATPs with anode as terminal electron acceptor. Other ways to bypass NADH reducing equivalents is the use of nanowires which can transfer electrons direct to anode (Gorby et al. 2006).

Eventually all reducing equivalents of carbohydrates are transferred to the anode and hence fermentations are not always detrimental to the anodic process. In fact they are essential as they convert carbohydrates into the substrates ideal for anodophiles to grow and generate electricity (Freguia 2010b).
2.3.5 Community Analysis

Pure cultures have been used to study specific aspect of MFCs such as the mechanism of transfer of electrons as it provides controlled environment. List of pure cultures used in MFCs are Actinobacillus succinogenes, Erwinia dissolven, Proteus mirabilis, Pseudomonas aeruginosa, Shewanella oneidensis, S. putrefaciens, Streptococcus lactis, Aeromonas hydrophila, Geobacter metallireducens, G. sulfurreducens, Rhodoferax ferrireducens, Klebsiella pneumonia (Das and Mangwani 2010; Du et al. 2007), Clostridium beijerinckii, C. butyricum, Desulfovibrio desulfuricans, Escherichia coli (Du et al. 2007). Although the most studied exoelectrogens, the gram-negative metal-reducing Shewanella spp. (Logan et al. 2005) and Geobacter spp. (Aelterman et al. 2008a; Jung and Regan 2007; Lee et al. 2003) are abundant in some MFCs inoculated with sediment and wastewater, respectively, their presence is not a necessity for high power production (Aelterman et al. 2006a; Rabaey et al. 2004). Hence, mixed consortia are preferred over pure culture as they are better suited in case of extreme environments provided by wastewaters due to synergistic association between microorganisms. For example, toxic metabolite is removed by one species helping the growth of the species preceding it (Ghazali et al. 2004). Also mixed culture provides higher stability during process disturbances and applicability of wider range of substrates (Rabaey and Verstraete 2005).

Different inocula such as anaerobic sludge (Rabaey et al. 2004), activated sludge (Lee et al. 2003), domestic wastewater (Liu et al. 2004), marine sediment (Bond et al. 2002) and soil (Ishii et al. 2008a), have been used to enrich electricity producing communities. The biofilm formed by use of these sources comprises of mixed bacterial cultures which are difficult to isolate. Our knowledge of microbial diversity and its role in nature is poor, mainly because traditional microbiological techniques, such microscopy and cultivation, have only a limited use for classification and identification of microorganisms. Classification of microorganisms on physiological and biochemical features is nearly impossible, because ~99% of all microorganisms in nature cannot be isolated in pure cultures mainly due to the ignorance of the culture conditions under which these microorganisms thrive in their natural environment. Therefore, for better understanding of microbial diversity and its role in ecosystem maintenance, other techniques, which complement the microbiological approach, are necessary.
2.3.5.1 Denaturing Gradient Gel Electrophoresis

Denaturing gradient gel electrophoresis (DGGE) is a standout amongst other electrophoretic techniques and has turned into a crucial and standard strategy for characterizing the microbial population in environmental microbiology. First step is to extract the total DNA present in the mixed bacterial sample. The next step is to amplify 16S rRNA gene using polymerase chain reaction (PCR). Then the amplification products are separated on a denaturing gradient gel based on DNA sequence. The denaturing chemical breaks both strands of DNA and separation takes place due to gradient formed. DNA segments with high GC content segments require a greater concentration of the denaturing chemical before the DNA strands break apart and migrate further down the gel. DNA segments with low GC content denature near the top of gel and stop migrating. DNA with the identical sequences migrates the same distance forming a band, thus band pattern generation directly reflects the genetic biodiversity of the sample. The number of bands is related to the number of dominant species. Coupled with sequencing and phylogenetic analysis of the bands, this method can give a good overview of the composition of a given microbial community (Sanz and Köchling 2007). The working principle is schematically represented in Figure 2.6.

![Figure 2.6 Schematic Representation of DGGE Working Principle (Ilyanassa 2012)](image)
In MFC studies, DGGE have been used to monitor the evolution of microbes. The DGGE technique involves amplification of 16s rRNA gene followed by identification (Lee et al. 2003). Form the literature, initially only two species of *Shewanella* and *Geobacter* were known to be electroactive, however, DGGE provided different angle to the analysis when a diversity of novel microbes appeared to be enriched in biofilms on anode. It was also observed that the diversity was dependent on the substrate and environmental conditions used in MFCs and until now no typical consortium has been confirmed from any clone library (Clauwaert et al. 2008; Logan and Regan 2006). Much remains unknown about the role and the interactions of microbes within both the electrode biofilm and the suspended communities, and in the contribution towards electricity generation. Understanding how microbial populations and biofilm structure evolve with time is essential if greater MFC power levels are to be achieved (Zhao et al. 2009).

Aelterman et al. (2008a) summarized different taxonomic classes present in MFCs which were Proteobacteria (64%), Firmicutes (13%), nonclassified sequences (13%) and Bacteroidetes (7%). They observed that though gram-negative bacterial species were present in most of the sequences, however, Firmicutes were vital part of these communities. Firmicutes have been dominant in MFCs fed with cellulose (Rismani-Yazdi et al. 2007), lactate (Borole et al. 2008) and acetate (Aelterman et al. 2006a; Wrighton et al. 2008; Xing et al. 2010). δ-Proteobacteria and Bacteroidetes were predominant on anode fed with glucose, lactate and acetate inoculated with anaerobic sludge (Jung and Regan 2007). MFCs inoculated with river sediments showed dominance of β-Proteobacteria, but α-Proteobacteria were observed when they were fed with glucose and glutamate (Phung et al. 2004). When wastewater was used as an inoculum, α-Proteobacteria, Bacteroidetes and Firmicutes were found to be most dominant communities (Xing et al. 2009).
2.3.6 Mechanism of Electron Transfer

Bacteria are so far known to transfer electrons to a surface via two mechanisms: electron shuttling via self-produced mediators (such as pycocyanin and related compounds produced by Pseudomonas aeruginosa (Rabaey et al. 2004; Rabaey et al. 2005b) and direct transfer e.g. by nanowires produced by Geobacter species (Reguera et al. 2005).

2.3.6.1 Direct Electron Transfer

In this mode of transfer, bacteria are attached to the surface of anode and transfer electrons directly through either conducting pilli-like structures called nanowires or bacterial cell surface receptors.

Nanowires

The conductive appendages (pilli-like structures) of both Geobacter and Shewanella species have been reported (Schröder 2007). These are called as bacterial nanowires. After the examination of the conductivity of nanowires, it was found that they carry electrons from the cell to the anode surface. Crucial respiratory cytochromes namely mtrC and omcA are assumed to be responsible for nanowires as mutants lacking those were found to contain non-conductive nanowires and also the mutants were impaired for their ability to reduce iron as well as production and electricity in MFCs (Gorby et al. 2006).

The production of conductive nanowires has been shown by microorganisms other than iron-reducing bacteria and also emerging evidence for interspecies electron transfer has been observed (Malvankar and Lovley 2012).

Cell-surface electron transfer

Nanowires are not the only mode of direct transfer of electrons. Certain microbes were found with surface blebs which are conductive points of contact between bacteria and anode (Logan 2008). Membrane-bound c-type cytochromes do exist in the cell membranes of few bacteria (Kim et al. 2002; Kim et al. 1999b) for the electron transfer. Multi-heme proteins have especially evolved in sediment inhabiting metal reducing microorganisms such as Geobacter (Lovley 2006) Rhodoferax (Chaudhuri and Lovley 2003) and Shewanella (Chang et al. 2006).
In their natural environment, iron (III) oxides act as the solid terminal electron acceptors, but in the case of MFC, the anode is used as the solid electron acceptor.

2.3.6.2 Mediated Electron Transfer

Mediated electron transfer has been proposed to occur via three different types of redox compounds: exogenous (artificial) redox mediators, microbially produced redox mediators or primary metabolites (Schröder 2007). Few examples are shown in Figure 2.7. Artificial mediators such as thionine, methylene blue and neutral red have been used to assist anodic electron transfer in MFCs (Ieropoulos et al. 2005; Park and Zeikus 2000). These mediators penetrate the bacterial cell and scavenge electrons from the reducing agents such as NADH. The reduced mediators then diffuse out of the cells and are oxidized on the electrode surface. Riboflavins and quinones released by *Shewanella oneidensis* and *Escherichia coli* have also been proposed acting as electron shuttles (Marsili et al. 2008; Qiao et al. 2008). The metabolic pathways of anaerobic respiration and fermentation produce metabolites that can also serve as MFC mediators. The sulphate/sulphide redox couple has been exploited e.g. in MFCs based on an anaerobic sulphate-reducing bacterium, *Desulfovibrio desulfuricans* (Cooney et al. 1996; Habermann and Pommer 1991). Sulfate is reduced by bacteria to hydrogen sulfide which in turn is abiotically oxidized on the anode electrode. Microbial fermentation products such as hydrogen, lactate and formate can be successfully oxidized on MFC anodes but catalysts are required on anode electrodes to facilitate the direct oxidation of these compounds (Rosenbaum et al. 2007; Zhao et al. 2005).

![Model for various compounds serving as electron shuttles between a bioelectrochemically active microorganism and the anode](Du et al. 2007).
Cyclic Voltammetry

The most common and useful electrochemical technique for the determination of the mechanism of electrode reactions underlying oxidation or reduction reactions is cyclic voltammetry (CV) (Zhao et al. 2009). CV is a simple technique and results are obtained in a relatively short time. CV requires a three-electrode system to obtain accurate results. In brief, the three electrodes are working, counter and reference. The potential is applied across the working and reference electrode and the flow of current is measured between working and counter electrode. CV is used to show whether a chemical system under study is reversible or irreversible. In MFC studies, CV experiments have been used extensively to: (i) investigate the mechanisms of electrode reactions involving both direct and indirect electron transfer between the biofilm and the electrode; (ii) determine the redox potentials of the chemical or biological species involved at the anode and (iii) to evaluate the performance of the catalysts being studied (Zhao et al. 2009). Generally forward and backward voltage scans are used with rates in the range of 1 – 100 mV/sec. Multiple peaks in the cyclic voltammograms of bioelectrochemical system may be observed due to multistep mechanisms, or the presence of several different redox species.

Some CV-based MFC studies showed characteristics of inter-conversion between active and inactive states, which is not an expected behavior of cytochromes (responsible for direct transfer of electrons) but is similar with that of enzymes (e.g. hydrogenase) (Vincent et al. 2007). For complex systems, where there are many unknowns especially in case of wastewaters, background experiments with blank electrolyte and anode (devoid of biofilm) are mandatory for determining the mechanisms of electron transfer.